animal, since part of the cooling period was passed while the animal was unconscious. This procedure was instituted because in exploratory experiments the animals which were cooled for a long time exhibited both struggling and shivering. The performance of these animals in the bell jar was poorer than their uncooled littermates, and much poorer than those in which cooling took place for the most part while the animals were unconscious.

From these five typical experiments it appears that the effects of reduction of colonic temperatures on one-day-old guinea pigs subjected to anoxic anoxia is to prolong life at about the same rate as might be expected from Van't Hoff's rule—i.e., about three times for the first 10° C differential.

Additional experiments, confirming the findings reported here and extending them to include also nembutalized animals, will be reported later when the statistical analyses of these data are complete.

It is concluded that elevation of body temperature is deleterious to day-old guinea pigs subjected to anoxic anoxia. Reduction of temperature, at least within the limits reported here, is beneficial when cooling is accomplished with minimal motor activity and shivering.

The Genera of Amoebae

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In recent years there has been considerable discussion about the correct scientific names of certain fresh-water amoebae. The recent articles by King and Jahn (*3*) and Wilber (*5*) include references to other work on the subject.

The three species concerned are: (I) *Amoeba proteus* Leidy (*Chaos diffluens*), (II) *Chaos chaos* Schaeffer (*Pelomyxa carolinensis, Amoeba carolinensis, Chaos carolinensis*), and (III) *Pelomyxa palustris* Greeff.

The controversy embraces two separate questions: one about the relative historical validity of the generic names *Amoeba, Chaos* and *Pelomyxa.* To this question we have no contribution to make. The other question is whether (I) and (II), or (II) and (III) should be placed in the same genus, or whether they belong to three separate genera.

Since the morphological criteria seem to be insufficient to allow a clear decision of the issue, we have tried to supplement the morphological evidence with a biochemical comparison of the three organisms.

Organisms (I) and (II) are readily available and their morphology and physiology is comparatively well known. Organism (III) is, at least in Denmark, comparatively rare, and in spite of persistent search we were able to obtain only four specimens. This limited material forced us to restrict our investigation to a few characteristic properties easily determined with the micromethods at our disposal. We decided therefore to determine the contents of two proteolytic enzymes, catheptic proteinase (substrate caseinogen, pH 4.0) and peptidase (substrate alanyl-glycine, pH 7.4). In addition we measured the approximate total protein content colorimetrically by means of the Folin-Ciocalteau reagent against a tyrosine standard. Details of these methods are described in a recent publication from this laboratory (*2*). As a basis of comparison we chose the cell volume rather than weight as suggested by Zeuthen (*6*), since the cytoplasm of *Pelomyxa palustris* (III) contains a great deal of foreign inclusions, e. g., grains of sand, which could be expected to falsify the density much more than the volume.

Organism (III) was identified as *Pelomyxa* in the following way: all four specimens were examined while alive with a water immersion objective giving 40 times magnification. The sand grains coating the surface and enclosed in the cytoplasm; the broad, slowly forming pseudopodia; the vacuolar appearance; and the bacteria in the cytoplasm, were easily seen. One of the specimens was crushed between cover slips and showed numerous nuclei about 10 μ in diameter, and "glycogen bodies" to 5–30 μ in size, which varied in shape from spherical to ellipsoid, some being quite irregular. Another specimen was fixed, sectioned, and stained. The details of bacteria, nuclei, and "glycogen bodies" which are described by Leiner (*4*), were confirmed. These are the characteristic features used for determining the species *Pelomyxa palustris,* and all of them are quite different from the corresponding features in *Amoeba proteus* (I) and *Choas chaos* (II). The third specimen was used for preliminary enzyme measurements, and the last and largest animal (total volume 6.5 μl) was used for final analyses in triplicate. The calculations in Table 1 for Species (III), therefore, all refer to this one specimen but they were roughly checked by preliminary experiments which indicated the order of magnitude of enzyme activity.

The values for species (I) and (II) are averages for a great number of individuals. The values for species (III) are the means of triplicate analyses. All values refer to a standard volume of 1 μl of cytoplasm. For

TABLE 1

	Protein	Peptidase	Proteinase	Peptidase / proteinase
	μg tyrosine	0.06 N HCl/hr	arbitrary units	
Species (I) *A. proteus*	4.0	53	43	1.2*
Species (II) *Ch. chaos*	2.6	62	28	2.2*
Species (III) *Pelom. pal.*	1.0	0.31	0.25	1.2*
Ch. chaos / *A. proteus*	0.65*	1.2*	0.65*	
Ch. chaos / *Pelom. pal.*	2.6*	200*	112*	

* These figures give ratios.

species (I) this volume was calculated on the basis of Chalkley's (*1*) measurements which give an average value of 0.15×10^{-3} µl per individual. For species (II) the volume was calculated from reduced weight measurements, asuming the relation 1 µg r. w. = 50 µl volume [Zeuthen (*7*)]. The volume of species (III) was calculated from direct measurements of dimensions.

As the Table shows, in organisms (I) and (II) the magnitudes measured correspond as closely as can reasonably be expected for intimately related species. As a matter of fact, the differences fall within the range of individual variations which can be found in one species. Organism (III), on the other hand, is decidedly different from the two others, at least with regard to the content of the two enzymes. This is most clearly seen by a comparison of the ratios given in the lower half of the table.

It might be thought that the remarkably low enzyme content of species (III) is not real, but rather due to inactivation during the homogenization prior to enzyme determination. However, this would not impair the validity of our comparison, since the homogenization and enzyme determinations were carried out exactly alike in all cases. Our figures thus indicate either a difference in enzyme content or in enzyme stability. Both properties ought to be equally suitable for the purpose of species comparison.

The result of our study is thus quite unambiguous: if biochemical features are considered as valid as morphological ones, organisms (I) and (II) ought to be placed in the same genus, while organism (III) seems rather different from the two others. Since nobody questions the validity of the name *Pelomyxa palustris* for species (III), we suppose that our results would support either the generic name *Amoeba* or *Chaos* for the other two. Not being taxonomists, we venture no suggestion as to which of these it should be.

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Separation of Chloride Group Anions by Partition Chromatography on Paper

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The separation of cations by partition chromatography was described in two previous papers (*3, 4*). These separations were only possible when hydrochloric acid was added to the solvent. This prevented both hydrolysis and the existence of ions in complex and simple form at the same time. The tailing or "comet" effect of Lugg and Overell (*5*) was thus avoided.

Westall (*7*) first measured the R_f value of the Cl^- ion in several solvents and observed that the addition of NH_3 to phenol inhibited the "comet" effect and gave Cl^- ions a constant R_f value, irrespective of the accompanying cation.

Partridge (*6*),, in a correction to his paper, observed that both Br^- and I^- travel at different speeds and interfered with the technique he used for the separation of sugars; but he did not give their R_f values or describe the shape of the spots obtained.

In this paper a new micromethod for the separation of Cl^-, Br^-, I^-, and CNS^- will be described. The solvent employed in the chromatograms is butanol saturated with I · 5N NH_4OH. This keeps the halides completely in the ionic form and appears to prevent any adsorption on the filter paper.

Separation is a simple matter in this method when compared with the technique required to show up the spots. After a trial of several techniques the simplest was found to be a method by which the paper was sprayed successively with two reagents and then washed and exposed to H_2S vapors.

The first method to be tried was an adaptation of that of Feigl (*2*). The silver chromate paper described there was prepared and the dried paper chromatogram was laid on top of it and sprayed with water. The halides were washed out and produced yellow spots on the red paper. This method was abandoned because the spots frequently moved on spraying with water, and also because the adsorption of halide ions on the paper might lower the sensitivity of the test.

The next method tried was spraying the chromatograms with silver nitrate solution, and then exposing to sunlight. This was found highly unsatisfactory, since a dark background formed which made the spots indistinct.

The most satisfactory method is as follows:

The paper is first sprayed with a mixture of ferric nitrate solution and hydrogen peroxide. The spraying is started from the liquid front and carried to within 4 cm of the starting point. This produces a red spot for CNS^-, R_f value = 0.42–0.48; and a blue spot for I^-, the R_f value being 0.29–0.32.

The whole of the paper is then sprayed with 0.1 N $AgNO_3$ solution, which discolors the spots originally formed and precipitates all four ions as insoluble silver salts inside the filter paper. This paper is now washed with very dilute HNO_3, so as to remove the excess of Ag^+ ions, two washes being sufficient. The paper is then held over H_2S and black AgS is formed wherever silver halides were precipitated. Chloride thus produces a brown spot (R_f = 0.1–0.11), and bromide a brown spot (R_f = 0.15–0.18).

In this paper the complete technique used will be described in detail, since a number of simplifications have been made to the method of Williams and Kirby (*8*), on which it is fundamentally based.

The aqueous phase is placed on the bottom of a 5-gal crock, where there is also a large Petri dish containing butanol. In the Petri dish is placed a measuring cylinder, fitted with a cork carrying a T-piece.